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Dose-Rate Effects on Lymphocyte Survival

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Dose-rate/Lymphocytes/Membranes

Lymphocytes isolated from bovine blood were irradiated at doses of 0.5, 1.0 and 1.5 Gy with dose-rates of 0.361 0.150, 0.062, and 0.006 Gy per min. The amount of trypan blue negative cells were counted directly after radiation and eight hours after the onset of the radiation, and compared with control cells. A considerable decrease of cells was found especially at the lowest dose-rate. At this low dose-rate, radiation induced cell loss already occurred during the irradiation period. These experiments suggest that the observed inverse dose-rate effects on lympocyte survival are caused by radiation induced damage to the plasma membrane of the cell. This phenomenon is especially pronounced at the lower range of dose-rates and is interpreted as a result of slowly progressing chain reactions occurring in the membranes, initiated by ionizing radiation.

INTRODUCTION

It has been shown by several authors that radiation induced peroxidation of unsaturated fatty acids is inversely dose-rate dependent[1-5]. Experiments in our laboratory have shown that in model membranes prepared from phospholipids extracted from cellular membranes, especially the polyunsatured fatty acids eicosatetraenoic acid (20 : 4) and docosahexanoic acid (22 : 6) are peroxidized in an oxygen dependent and dose-rate dependent way[5]. It has also been shown that biomembranes from liver cells isolated from mice which were whole body X-irradiated at different dose-rates, had a higher peroxidation capacity when the irradiation was performed at lower dose-rates[6]. It is generally known that sparsely ionizing radiation is less effective in the cell killing of dividing mammalian cells, when the dose is delivered at a low dose-rate. This is attributed to repair of sublethal damage during the exposure[7]. Lymphocytes normally do not die a reproductive death after irradiation but are mostly killed in interphase. Lysis of these cells after a radiation insult might be caused by damage to the plasma membrane. If lipid peroxidation in the plasma membrane is the predominant damaging process in lymphocytes irradiated at low dose-rates, than an inverse dose-rate dependency of radiation induced lymphocyte lysis may be expected.

The present short communication reports on preliminary experiments which indeed point to a lower lymphocyte survival after X-irradiation at low dose-rates.

MATERIALS AND METHODS

The lymphocytes used in these experiments were obtained from freshly drawn heparinized bovine blood. The blood was diluted 1:1 with 0.14 M NaCl (20°C) and brought on top of a 4 m*l* cushion of Ficoll-Paque (Farmacia, Sweden) in portions of 5 m*l* in 15 m*l* siliconized glass centrifuge tubes. Centrifugation was done at room temperature in a Sorvall GLC-2 centrifuge at 1800 rpm for 20 minutes. The band of white cells laying on the Ficoll material after centrifugation was isolated, suspended in 10 m*l* 0.14 M NaCl, centrifuged again for 5 minutes at 1000 rpm in the same centrifuge, and resuspended in a balanced salt solution consisting of 0.126×10^{-2} M NaCl, 5.4×10^{-4} M KCl, 5×10^{-6} M $CaCl_2$, 9.8×10^{-5} M $MgCl_2$ and 14.5×10^{-3} M Tris HCl pH 7.6. In order to remove adherent cells (macrophages, granulocytes)from the lymphocyte preparation a 2 hours incubation at 37°C in petri dishes was performed. The non adherent cells were counted in 0.1% trypan blue in a Bürker Chamber. At this point the lymphocyte cell density was adjusted to 5×10^6 cells per m*l* and divided in 2 m*l* portions in 15 m*l* siliconized glass test tubes.

X-irradiation of the lymphocytes was carried out at 37°C with a Philips Müller MG 300 X-ray machine operated at 200 kV and 5 mA. The beam was filtered by 0.5 cm Cu and 0.5 mm Al. The different dose-rates were obtained by changing the focus-object distance. Measurement of the dose-rate was done with an ionization chamber calibrated against a standard radium source. Mostly three different dose-rates were applied at a constant dose to the same lymphocyte suspension.

RESULTS

Radiation experiments with different deses and at various dose-rates were performed and cell counting in 0.1% trypan blue was done directly after irradiation and 8 hours after the onset of the irradiation. Care was taken that the unirradiated control cells received exactly the same treatment. Results are expressed as a percentage of unirradiated controls which were counted at the same time.

Dose-rate effects could already be found directly after irradiation. This is illustrated in Table 1. For each dose used a reduced amount of viable cells is present at the dose-rates of 0.062 Gy/min and 0.006 Gy/min. No immediate radiation effect on cell viability was observed at the dose rate of 0.361 Gy/min and a very small effect at 0.150 Gy/min. A considerable standard deviation (SD) was inevitable in these experiments. In Table 2 the situation after an eight hours incubation period is shown. Also here the data are expressed as percentage of control cells which had been incubated for the same total time. From this table it can be learned that almost no additional radiation damage had been developed in the post irradiation incubation period for the cells which had been irradiated at the two lowest dose-rates. This is not the case for the two higher dose-rates. As compared to the control cells, at this definite eight hours time point, some radiation induced damage for the higher dose rates has devel-

Table 1
Survival of lymphocytes as measured directly after X-irradiation at different dose-rates.

Dose-rate (Gy/min)	Amount (%) of viable cells after various doses		
	0.5 Gy	1.0 Gy	1.5 Gy
0.361	99±10	102±11	96± 8
0.150	94± 8	91± 9	89± 7
0.062	87±10	77± 7	71±12
0.006	58±11	42±10	30±11

Cell counting was performed immediately after the irradiation and compared with non irradiated control cells which had been incubated for the same time and under the same conditions. Results are expressed in terms of trypan blue negative cells, as a percentage of unirradiated controls. Data are means of at least three replicates±SD.

Table 2
Survival of lymphocytes, 8 hours after the onset of X-irradiation.

Dose-rate (Gy/min)	Amounts (%) of viable cells after various doses		
	0.5 Gy	1.0 Gy	1.5 Gy
0.361	96±13	92± 8	88±14
0.150	84± 9	81±10	78± 8
0.063	77±12	76± 9	71±14
0.006	53± 7	43±11	28± 8

All data are expressed as a percentage of the unirradiated controls which were also incubated at 37°C for 8 hours. As a result of the incubation the amount of control cells had decreased to 72% of the starting material (5×10^6 cells per ml). Conditions as in table 1.

oped during the post irradiation incubation period. The dose rate effect was present to about the same extend after longer incubation periods (not shown).

DISCUSSION

The experiments prsented here show that most of the irradiation induced cell loss at low dose rates takes place during the irradiation period. The dose-rate effects observed cannot be attributed to incubation artefacts because postirradiation incubation (almost eight hours for the two higher dose-rates as shown in Table 2) cannot eliminate the differences in cell loss.

These results are in contrast to what is normally found for cells dying a reproductive death after irradiation. Lymphocyte cell survival, as shown here is inversely dose-rate dependent. This observation suggests that at low dose-rates (<0.06 Gy per min) lymphocytes die (lyse) because of damage to the plasma membrane. More experiments are necessary to precisely determine those dose-rates where the membrane

phenomena become manifest. Below a certain threshold of dose-rates, lipid peroxidation in these membranes apparently can no longer be efficiently inhibited. Inhibition of lipid peroxidation in biomembranes can be caused by antioxidants[8,9]. It has been shown that more α-tocopherol is needed in the membranes to protect polyunsaturated fatty acids against radiation induced lipid peroxidation when low dose rates are applied[5].

The results shown in the current report suggest that at low dose rates a totally different mechanism is reponsible for lymphocyte cell killing (interphase death) than for induction of chromosome aberrations. It has been reported by several authors that more chromosome aberrations occur in lymphocytes after irradiation at higher dose-rates. In a recent study of Bauchinger *et al.*[10] dose rates and doses of Co γ-irradiation were used which were in the same range as applied in the current study. Also these authors found a higher yield of dicentrics at higher dose-rates. Knowledge of dose-rate effects in different cell types and under different conditions is not only important in risk estimation after ionizing radiation but is also helpful to gain insight in the possible molecular mechanisms of radiation damage in mammalian cells.

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